APPLICATION OF CRITICAL FLUID TECHNOLOGY TO NUTRACEUTICAL EXTRACTION AND REFINING

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ABSTRACT

Critical fluid technology has, and will play in the future, an important role in the processing of natural products for nutraceutical ingredients. This view is based on the perception that consumers of nutraceutical products will demand "natural" products that are devoid of organic solvent residuals, and superior in strength and effectiveness. Processing media such as carbon dioxide, water, and ethanol; under pressure, offer a safe and effective way of extracting, refining, and concentrating extracts for this market. In this review, an advocacy is made for the use of these "natural agents'' to extract and fractionate nutraceutical ingredients ranging from lipophilic oils to more polar moieties derived from herbs.

Examples are provided of typical processing equipment and options that can be applied for processing natural products and handling of solid and liquid feed materials. These include bench scale equipment as well as scaled up modules for pilot plant service. In addition, an assessment is made as to the state of production facilities for conducting critical fluid processing in the world. The role of analytical scale extraction and chromatography for developing processing schemes and conditions in characterizing fluid-derived extracts is delineated. Extraction and fractionation schemes for enriching and purifying nutraceutical preparations are discussed with examples (tocopherols and phospholipids). A historical and future perspective is offered on key nutraceutical products that can, or could be produced by these unique and environmentally benign processes.

KEYWORDS: critical fluid, extraction, fractionation, nutraceutical, supercritical

There is a certain synergy that exists between critical fluid processing and nutraceuticals. Consumer use and acceptance of these natural products is heightened by the appeal that they have been "naturally processed". However, this is not a new development and it is interesting to note that many of today's nutraceutical components have already been extracted or separated using the above-named natural agents. In addition, critical fluid processing has also been used to create healthier low-fat foods, such as: eggs with a reduced cholesterol content (Froning et al., 1990), low-fat nut products (Passey, 1994) and fat/cholesterol reduced meats (King et al., 1993). However, more recent food product trends show that the focus has shifted from removal of undesirable components such as cholesterol to addition of beneficial components to formulate "functional foods" with health benefits (Sloan, 1999). Extraction and refining of nutraceuticals from natural sources using critical fluid processing to be sold in pill, powder or extract form or to be incorporated into functional food formulations is rapidly growing. There is no doubt that the development of such "natural" products coupled with a proper marketing campaign provides a powerful stimulus for the consumer to try them.

1.2. Choice of critical solvents

SC-CO_2 with a critical point of 31°C and 73 atm reigns supreme as the principle-processing agent in critical fluid technology. However, like any other extraction solvent SC-CO_2 cannot be effectively utilized for all tasks, and is a poor solvent for polar compounds. For certain applications, the addition of a minimal amount of a polar cosolvent (usually an organic solvent with a critical temperature, T_c, higher than CO_2), suffices to improve the extraction of targeted components from a natural product matrix; although the number of GRAS (Generally Regarded As Safe) cosolvents suitable for this purpose is rather limited (e.g., water, ethanol, acetic acid). Cosolvents can be used in conjunction with SC-CO_2 either in a single or multi-step extraction process to produce the desired end result.

Propane has also been evaluated for critical processing of lipids since it has a lower critical pressure (P_c=48 atm, T_c=97°C) than CO_2 and the solubility of lipids in near-critical propane is higher than that in near-critical CO_2 (Straver et al., 1998). A CO_2+propane mixture has been used for de-oiling of lecithin and fractionation of glycerides (Peter, 1996). However, despite its higher critical pressure, CO_2 seems to be more acceptable to the food industry than propane because it is inflammable.

Other alternatives, which embrace the green processing concept, are the use of liquefied gases (e.g., LCO_2) and pressurised liquids. Liquids under an appropriate external pressure will not boil at extraction temperatures above their boiling point, and can produce superior results in specific cases, compared to conventional liquid solvent extraction (Clifford et al., 1999). For the processing of natural products, the selection of extraction temperature is critical to avoid degradation of the extracted as well as residual components depending on which is of interest. Liquefied gases, such as LCO_2 are used at near- or sub-ambient temperatures (Schultz and Randall, 1970; King and Bott, 1993; Brogle, 1982), thereby avoiding conditions conducive to thermal degradation of the extracted components. Currently, these fluids are being re-examined in lieu of supercritical solvents.

Perhaps the pressurised liquid that attracts the most interest is subcritical water. Its phase diagram (Figure 1) is in general similar to that of CO_2, except for the fact that the phase boundary between solid and liquid phases has a negative slope while that for CO_2 is positive. In addition, the critical temperature and pressure of water are much higher (T_c = 374°C, P_c = 221 atm). However, there is a region of opportunity for water in its subcritical state that lies between 100 - 200°C, in the liquid region defined in Figure 1, between 1 and approximately 100 atm. A considerable literature exists on the properties and use of water in the superheated state (Clifford and Basile, 1998), and temperature is the key parameter for regulating the

solvent power of superheated water (Hawthorne et al., 1994). The dielectric constant of water, which varies inversely with temperature, ranges approximately from 30-60 over the temperature range of 100 - 200°C. Dielectric constants of this magnitude are in the range of those exhibited by polar to moderately polar organic liquids.

Subcritical water can potentially be used as a substitute for less-desirable organic solvents to extract and process natural products. Recent studies have shown that subcritical water can be effective for extraction of natural products such as cloves (Clifford et al., 1999) and rosemary (Basile et al., 1998). However, such applications require further investigation to evaluate the extent of hydrolysis and degradation that may occur under such extraction conditions. As well, quality and composition of extracts need to be compared to those obtained with SC-CO_2 and SC-CO_2+cosolvents. Moreover, removal of water from the final product also requires the application of heat, which needs to be minimised for any natural product application.

Figure 2 summarises an "all natural" approach to processing natural products for nutraceuticals. On one end of the solubility spectrum lie SC-CO_2 and LCO_2, while pressurised water on the other end is available for isolating polar moieties. Several combinations of GRAS cosolvents can be coupled with CO_2 for cases where this approach proves viable. Such a spectrum of solvents creates an opportunity to develop new extraction and fractionation processes for natural products.

2. PROCESS DEVELOPMENT AND EQUIPMENT

The development of a suitable critical fluid-based process for isolating a nutraceutical ingredient takes place in several sequential steps of increasing complexity and scale up. These can be summarised as follows:

- Bench scale evaluation of process feasibility
- Pilot plant evaluation stage
- Scale up to production plant stage

Several examples of equipment appropriate for conducting extractions and fractionations with critical fluids at each step of process development are provided.

2.1. Bench scale evaluation

Bench scale evaluation of the feasibility of extracting a substance is usually accomplished with the aid of a small-scale extractor. Such units are available commercially, but an increased experimental flexibility is achieved by constructing home-built units. Alternatively, small-scale supercritical fluid extraction (SFE) equipment, normally intended for analytical uses of critical fluid technology, can also be used to assess and optimize the extraction of a natural product. Both of the above approaches can also incorporate the introduction of a cosolvent into the critical fluid as necessary.

Figure 3 is the schematic of a simple, bench top extraction system that has proven very versatile in our laboratories. A fluid source (e.g. CO_2), A, can be either gaseous or liquefied, the latter being more commensurate with what is used in scaled up equipment. A compressor or liquid pump, C, delivers the fluid through a tandem switching valve, SV-1 and SV-2, to a tubular extraction cell, that is thermostated to maintain the appropriate critical state (sub- or supercritical). The fluid passes through the cell containing the material to be extracted and is passed on through a similar switching valve arrangement into either a micrometering valve, MV or back pressure regulator; where the pressure of the fluid is reduced. Back pressure regulator or metering valve arrangements for fluid pressure reduction are heated to overcome the attendant Joule-Thomson cooling effect before the depressurized fluid is sent to the collection vessel. The so-called receiver vessel can consist of several formats and may be

packed with internals or kept at a very low temperature (-15°C) to eliminate entrainment of the extracted components in the rapidly expanding critical fluid. Gas flow under ambient conditions is assessed with the aid of a flow meter, FM, and fluid totalizing module, GT. The described extraction unit can be reformatted for different types of fractionation experiments involving critical fluids and has proven extremely flexible at a modest cost in our laboratories (King, 1995).

Bench scale equipment for preliminary evaluations are manufactured by such companies as Autoclave Engineers, Fluitron, Nova Swiss, Mueller, Nova Sep, Thar Designs, Pressure Products, Inc., Supercritical Fluid Technologies, Marc Sims, Inc., and Separex. Some commercial units may be limited with respect to the achievable pressure range, fluid flow rate, and scale of the extraction cell (King, 1995). However, it is also possible to make design modifications on some bench-scale commercial units by experienced users to meet various objectives, such as addition of a cosolvent pump. Making changes on larger commercial units is not recommended without consulting the manufacturer.

2.2. Pilot plant scale

The pilot plant evaluation stage incorporates both a scale up of the previously described approaches as well as several other processing options. Although somewhat rare, single pass (with respect to the extraction fluid) pilot and production plants are known to exist, but plants designed for fluid recycle with integrated heat exchangers are much more the norm. Recycling the critical fluid economizes on the mass of solvent required to carry out the extraction.

Batch pilot and production scale plants consisting of a single extraction and a single separator vessel have been in operation for some time. Such an approach may be feasible if the product is of very high value. However, downtime associated with pressurization/ depressurization and loading/unloading of a batch of solid material into a single extraction

vessel may be significant depending on system design.

A semi-continuous operation is possible with the incorporation of several extraction vessels. Such a pilot plant (King, 1997) consisting of three 4-L vessels and solvent recycle capability is shown in Figure 4. Use of 4-L vessels permits multi-kilogram quantities of natural products to be extracted. Units with multiple extraction vessels require appropriate valving and tubing to allow several modes of operation. Carbon dioxide, the extraction solvent, can be routed sequentially to one or more of the extraction vessels, which can be operated such that one of the vessels, A, is being extracted while another vessel, B, is being loaded with the solid material to be processed, and a third vessel, C, is undergoing pressurization/depressurization. The collection vessel must be of sufficient size to allow separation of the extracted solutes from the depressurized fluid. In concert with the 4-liter extraction vessel pictured, a 2-liter collection vessel has proven sufficient for most purposes. A sorbent-laden column maybe inserted in the low pressure side of the solvent recycle line for purifying the fluid of any unwanted odoriferous volatile compounds. A provision is made for makeup of fluid lost upon draining the extract from the collector vessel.

Other types of pilot plants, including commercial units embracing this principle of semi-continuous operation are available. Some selected vendors of pilot plants; although this is not an exclusive list, include Krupp, National Forge, Glitsch, Steelcraft, UHDE, Chematur, Colin Ryder Ltd., and Separex.

Continuous feeding of solid materials into a high-pressure extraction unit will improve the efficiency of the operation significantly, but it is a major challenge. Several novel approaches are being evaluated at the pilot plant level. Eggers et al. (1985) described the use of a screw press to obtain pressed oil from an oilseed and to feed the press cake continuously to the top of a high-pressure extractor where it is contacted countercurrently with SC-CO_2. A second

screw conveyor removes the extracted meal at the bottom of the extractor. Another approach to continuous solids handling makes use of an extruder as an extractor after some modifications to allow the critical solvent to be introduced into the extruder barrel. Thus, a truly continuous countercurrent extraction of solids can be accomplished. In addition, introduction of SC-CO_2 carrying nutraceutical ingredients into the extruder head allows "impregnation" of a natural product matrix with the active components as it leaves the extruder. Two active research laboratories on utilization of extruders in critical processing are the Praxair Corporation in Chicago, Illinois and the Department of Food Science at Cornell University, respectively.

Critical processing of liquid materials for fractionation purposes is usually carried out in a packed column, which may incorporate a temperature gradient along its length (Nilsson, 1996), as shown in Figure 5. This system can be operated on a continuous basis by the use of a pump to feed the liquid substrate to be fractionated at the bottom, top or a central position in the column to achieve a co-current or countercurrent operation mode. Components to be separated in the feed are subjected to a thermal gradient, where each of the designated zones for various sections of the column, have an increasing temperature in the sequence T2, T3, T4, and T5. This creates an internal reflux producing an enrichment section toward the top of the column and a stripping section in the cooler section toward the bottom. Thus, components in the feed material are fractionated based on their relative solubilities in a ever decreasing CO_2 density gradient, as well as their respective increasing vapour pressures as they ascend the column. It is also possible to add an external reflux pump to such a system. Such columns are not available commercially and have to be custom built either in-house or by manufacturers of extraction units.

A recent novel development for continuous extraction/fractionation of liquids with critical

fluids makes use of a microporous membrane instead of a packed column (Robinson and Sims, 1996; Sims et al., 1998). This "porocritical" fluid fractionation process is depicted in Figure 6 and shows "near" critical CO_2 being fed into a concentric tube arrangement within a pressurized module, countercurrent to the flow of aqueous liquid feedstock, which is being pumped internally through a microporous membrane. Separation is driven by the concentration gradient of the solute across the membrane. CO_2 is recycled after separation of the extract. Even though this process is used for extracting natural flavors and fragrances, it shows potential for enrichment of nutraceuticals in liquid streams.

2.3. Production plant scale

Final scale up to the production plant stage is a serious undertaking, both with respect to mechanical complexity/safety and economics. Such plants have traditionally been permanent fixtures, however there is a trend toward increased flexibility (e.g., multiple use modules for extraction, fractionation and reaction), considering the initial investment in compressors and fluid sources. Portable extraction units have been described and demonstrated for fieldside processing to permit extraction of natural products commensurate with their harvest.

The purpose of this section is to provide a brief overview of the magnitude and scope of critical fluid technology as applied to industrial scale production. Information on critical fluid production facilities can be limited due to proprietary constraints, however industrial scale processes using supercritical fluids have existed for close to thirty years and critical fluid processing is no longer a "cottage" industry. These production facilities vary considerably in the magnitude of the operation, ranging from the large Houston-based coffee decaffeination plant of General Foods to small extraction facilities focussed on the processing of non-commodity items. A sizeable segment of the production facilities is devoted to the processing of hops. However, these facilities process hops for only part of the year and potentially have

additional capacity, which could be devoted to processing of materials having nutraceutical value.

In terms of handling the large quantities of solid material, most of the production plants operate on a semi-continuous basis with several extraction vessels as described previously (Fig. 4), even though some batch units with a single vessel are also in operation. General Foods decaffeination plant displays a unique approach to solids handling in a semi-continuous manner using a single vessel (McHugh and Krukonis, 1994). In this design, coffee beans are loaded and unloaded into two lock hoppers above and below the extraction vessel, respectively, with simultaneous pressurization and depressurization of lock hoppers only, while the extraction vessel continuously operates at high pressure. Such an extractor design can also be applied for the recovery of nutraceuticals from various plant materials. Another important aspect of solids handling for nutraceutical purposes is the pre-treatment of solids prior to extraction. Grinding of plant material is usually necessary to increase surface area and enhance extraction efficiency. Drying may be needed depending on the initial moisture content. In both cases, temperature control is critical to minimize overheating and degradation of active components.

The authors estimate that there are at least 32 plants worldwide devoted to critical fluid processing. Currently most of these are centered in Germany, the United States, France, and Japan. Nations, such as Britain, Australia, Canada, India, and Italy continue to develop more capacity for critical fluid processing; and other nations with a rich litany of natural products, will undoubtedly enter the marketplace as users. Table 1 shows a list, which is not inclusive, of many users of critical fluid extraction for food and natural product processing throughout the world. Key players in the various market segments are: decaffeination - General Foods, SKW Trostberg, Kaffee HAG, Hermsen; hops processing - HVG Barth (NATECO$_2$), John

Haas, Yakima Chief, Carlton United Breweries, Steiner Hops, English Hops, SKW Trostberg; flavors/spices - Cultor, Quest, Flavex, Norac, Ogawa, Fuji Flavor, Kobe, Mori Oil Mills, Takeda. Many of the processors listed under the generic heading of flavors/spices also produce a variety of other natural products such as specialty oils, natural pigments and antioxidants; e.g., Flavex is involved in the processing of ginseng among other moieties and Norac is processing rosemary antioxidants, saw palmetto, kava kava and other botanicals (Nguyen, 1999). As noted above, many of these companies have additional capacity for fulfilling the needs of the nutraceutical market and other firms, on a more diminutive scale, are preparing to enter the market focussing on the processing of niche natural products. Some of the new players addressing nutraceuticals processing are KD-Pharma - IQA, Wells Investments Ltd., and Aromtech - OY

There also exists a cadre of companies that specialize in research and development and toll refining up to a certain scale. Among the more prominent ones are Phasex, Praxair, Norac, Marc Sims Inc., Separex - Hitex, Flavex, Express Separations, Bradford Particle Design, CPM Inc., Wells Investments Ltd., and Aphios Corp. These companies and a host of other organizations; consultancies, academia, and government laboratories, can be useful sources for technical consultation/information.

What is a typical extraction/processing plant like in terms of scale? This is hard to quantify without picking what might be a "typical" example, but it is worth citing the NATECO$_2$ production facilities in Wolnzach, Germany as an example of a technically sophisticated and diverse operation. Some of their stated capacity and capabilities are as follows:

Production Plant #1 - 4 X 2,000 L Extraction Vessels

Laboratory Plant #2 – 1,000 mL Countercurrent Column

Production Plant #3 - 4 X 4,000 L Extraction Vessels

Production Plant #4 - 3 X 500 L Vessels

Pilot Plant #5 - 2 X 200 L Countercurrent Column

This plant has historically focussed on the processing of hops but has diversified over the years to include the processing of other natural products, thereby providing for maximum utilization of the plant facilities.

A special facility that deserves some mention is the relatively new KD-Pharma/Industrias Quimicas Asociadas plant for producing concentrates of ω-3 fatty acid esters (eicosapentaenoic, EPA, and docosahexaenoic acids, DHA) from fish oil in Tarragona, Spain. This production facility employs a combination of SFE and supercritical fluid chromatography (SFC) to produce fish oil ester mixtures of 95% purity. The process uses a proprietary silica-based packing in the SFC stage to separate the ω-3 fatty acids from the ω-6 moieties, as described in detail by Lembke (1998) for the pilot plant scale operation. Increasing the EPA content of this natural product improves its nutraceutical functionality and the resultant product competes favorably against those fish oil concentrates derived via competitive processes. Interestingly, the basic fractionation concept was derived from research performed on analytical scale SFC columns.

3. PROCESSING SCHEMES AND NUTRACEUTICAL APPLICATIONS

3.1. Extraction

Numerous examples of SC-CO_2 extraction of various plant materials from around the world are available in the literature and it is beyond the scope of this chapter to provide a comprehensive review of all the nutraceutical or potential nutraceutical products that could be processed with critical fluids. However, extraction of specialty oils and carotenoids will be highlighted. The focus in majority of these studies has been the optimization of

extraction temperature and pressure to maximize extract yield and selectivity for the compounds of interest. High CO_2 density is equivalent to high solvent power and thus higher yield. However, higher extract yield is generally associated with decreased selectivity for the desired compound(s).

Specialty oils receive attention as a nutraceutical and are sold in capsules due to their unique fatty acid composition and/or the presence of lipid-soluble minor components that were shown to have health benefits. Specialty oils also find applications in medicine and skin care products. Antioxidant activity leading to protection against cancer and reduction in serum cholesterol levels thus reducing the risk of cardiovascular diseases are some of the benefits attributed to the minor components. Oat and barley oils are rich in tocopherol and tocotrienol. Oat oil was extracted using SC-CO_2 by Fors and Eriksson (1990), but its tocol content was not analyzed. Barley oil was extracted from whole barley flour as well as pearling flour since the majority of lipids are located in the outer layers of the kernel (Temelli and Noosuk, unpublished data). Tocopherol content of the SC-CO_2 extracted oil from pearling flour was significantly higher that that of oil extracted with petroleum ether. Rice bran oil extracted with SC-CO_2 contained α-tocopherol, oryzanol and sterols (campesterol, stigmasterol, β-sitosterol) (Shen et al., 1996). Sterol content of SC-CO_2 extract of saw palmetto was ~4 times that of an ethanol extract (Nguyen, 1999). Evening primrose oil, which is a rich source of γ-linolenic acid, was extracted by Favati et al. (1991) at 197-690 atm and 40-60°C. Borage, blackcurrent, and flax oils are other sources of γ-linolenic acid, which have been extracted with critical fluids. Sea buckthorn oil was extracted from the berry's seeds and pulp with SC-CO_2 due to its high unsaturated fatty acid and vitamin content (Stastova et al., 1996). Other specialty oils that have also been extracted with SC-CO_2

are wheat germ, avocado, sorghum bran, and amaranth. As well, critical fluids were employed to obtain oil from fungi or marine sources, like spirulina, which are devoid of cholesterol (Hu, 1999). Various other botanicals and spices including chamomile, mint, rosemary, paprika, feverfew and gingko biloba (analytical studies), garlic, and ginger have been extracted by SC-CO_2 and some of these products are available commercially. Such plant extracts are enriched in various phytochemicals with health benefits (Anonymous, 1997).

Carotenoids, which are lipid soluble pigments, have antioxidant and provitamin (β-carotene) activity. β-Carotene was extracted from natural sources such as carrots (Barth et al., 1995; Vega et al., 1996), sweet potatoes (Spanos et al., 1993), and microalgae (Mendes et al., 1995) using SC-CO_2 with and without the addition of ethanol as a co-solvent. Vega et al. (1996) achieved 97% β-carotene recovery from carrot pulp residue of juice extraction at 70°C/272 atm using SC-CO_2+10% ethanol. Favati et al. (1997) extracted lycopene and β-carotene from by-products of the tomato industry and reported recovery of 80% and 70%, respectively, at 690 atm and 80°C. Carotene and lutein were also recovered from leaf protein concentrates (Favati et al., 1988).

3.2. Fractionation

Enrichment of a nutraceutical ingredient to a sufficient purity or strength may not be accomplished via a single extraction step. This will necessitate the use of a fractionation scheme. The ability to fractionate naturally derived materials in a benign way using critical fluids is of particular interest to processors of nutraceutical ingredients because the relatively pure nutraceutical components have higher added value. Several approaches can be taken to achieve this goal depending on whether the feed material is solid or liquid and specific examples of this processing mode will be cited below.

a) Fractional extraction

Fractions can be collected as a function of time throughout an extraction since composition of the extract changes over time. As small molecular weight components are depleted selectivity shifts towards larger molecules depending on the extraction conditions and CO_2 flow rate. For a more pronounced compositional difference between the fractions, extraction conditions can be changed to achieve increasing solvent density over time. Nilsson et al. (1989) fractionated fish oil ethyl esters by applying incremental pressure programming and obtained a fraction enriched in EPA. Zhao et al. (1987) collected fractions of rice bran oil at different pressures and showed that the free fatty acid content of the fractions obtained at higher pressure were lower. Such a low acid value fraction was critical since acid value of rice bran oils in general is higher than that of other oils due to high lipase activity.

Another approach to fractional extraction is to change solvent composition by addition of a co-solvent over time, similar to gradient elution in chromatographic separations. In this case, neutral components can be extracted first and polar components would be concentrated in later fractions. Dunford and Temelli (1995) and Montanari et al. (1999) applied this approach to recover canola and soybean lipids, respectively. Neutral lipids were extracted with SC-CO_2 first and then ethanol was added into CO_2 as a co-solvent and polar phospholipids were concentrated in the second fraction.

Fractional extraction of a natural substrate by the use of a combination of various "green" fluids is also possible as suggested by the hypothetical scheme offered in Figure 7 for soybeans. Here the non-polar components, such as neutral lipids, carotenoids, triterpenes, or phytosterols, would be preferentially removed by carbon dioxide followed by extraction with a

CO_2/cosolvent combination that has the potential to remove more polar components, such as phospholipids, phenolic acids or coumarins. Finally after removal of the above components, subcritical water could be applied to isolate the isoflavones, phytates, etc. It should be recognized that there will be some overlap of the targeted compounds removed by each solvent, but the scheme depicted in Figure 7 is both environmentally benign, and leaves behind a proteinaceous meal for further use that is devoid of objectionable solvent residues. The effect of subcritical water extraction on the functionality of residual proteins would require careful evaluation.

b) Fractional separation

A more efficient and rapid way of obtaining extract fractions from a solid matrix is by fractional separation. In this approach, extraction and fractionation are carried out simultaneously and the economics of the process is improved favorably. All components that can be solubilized in CO_2 are extracted from the solid matrix at a condition of very high CO_2 density. The extract is then fractionated in a series of separators where the conditions are adjusted for a stepwise decrease in CO_2 density. Extraction and fractionation of essential oils of numerous plant materials using this approach was reviewed by Reverchon (1997). Nguyen et al. (1998) described fractional separation of various spice extracts including ginger, celery and paprika where different phytochemicals were concentrated in the essential oil or oleoresin fraction. Such an approach combined with the use of 3-4 separators will result in fractions where phytochemicals of nutraceutical value are concentrated in different fractions.

c) Column fractionation

A continuous countercurrent packed column as described previously (Fig. 5) is used for

the fractionation of a liquid product. With the application of a temperature gradient along the column, an internal reflux can be created leading to enhancement of separation efficiency. Thus, the desired component may be concentrated either in the extract or the raffinate.

One of the simpler applications of column fractionation is depicted in Figure 8, which involves the countercurrent separation of phospholipids from soybean oil. High pressure carbon dioxide is fed into a pressure vessel packed with segmented gauze mesh packing (the "refining vessel" in Figure 8), where it travels upwards contacting soybean oil, which is pumped into the top of the refining vessel. The soybean oil is solubilized in the SC-CO_2 while the phospholipids being insoluble in the CO_2, descend to the bottom of the refining vessel. The oil can be recovered by lowering the pressure and temperature in the receiver vessel allowing recycle of the CO_2 back to the main compressor. By using this technique, a very rich lecithin precipitate can be rendered without the use of organic solvents (List et al., 1993). Similarly, critical fluid jet extractor-based methods can be used to produce high purity lecithin for the nutraceutical market (Stahl et al., 1987; Eggers and Wagner, 1993).

Continuous countercurrent packed columns (Fig. 5) have been used to fractionate various natural materials including fractionation of fish oil (Krukonis et al., 1992) or butter fat (Bhaskar et al., 1993). Processing palm oil in a packed column resulted in a refined palm oil in the raffinate stream with increased carotenoid content (1225 ppm) after removal of free fatty acids and mono- and diglycerides (Ooi et al., 1996). Brunner et al. (1991) used a packed column to fractionate deodorizer distillate from vegetable oil refining for enrichment of tocopherols. Similarly, squalene was recovered from olive oil deodorizer distillate (Bondioli et al., 1993) and shark liver oil (Catchpole et al., 1997) using SC-CO_2.

d) Chromatographic fractionation

Chromatographic fractionation using critical fluids as mobile phases has been studied for some time now, however scale up from the analytical regime has been less prevalent. Simulated moving bed technology has been incorporated into the chromatographic fractionation of stereoisomers utilizing critical fluids (Giese et al., 1999). Studies at the National Centre for Agricultural Utilization Research by King et al. (1996) have demonstrated that preparative SFC can be coupled advantageously with a selective SFE enrichment stage to yield oily concentrates rich in nutraceutical ingredients. As shown in the processing scheme in Figure 9, flaked soybeans are initially extracted at a relatively low pressure to enrich the components of interest, the tocopherols. This fraction is then moved sequentially on to a sorbent filled column, for further fractionationation to yield a tocopherol-enriched extract of nutraceutical value. The advantage of this approach is that it allows one to enrich a particularly valuable nutraceutical ingredient from a natural matrix, without contaminating the remaining matrix with a noxious agent. Enrichment factors relative to the tocopherol content in the original soybean flakes are tabulated in Table 2. Note that these are only modest for application of the single SFE stage, however significant enrichment of the desired components can be obtained by applying the chromatographic fractionation step. A similar approach was applied to the enrichment of phospholipids, as will be described later. Pure phosphatidylcholine and phosphatidylserine are finding widespread use, the latter in improving brain function, hence the challenge for those using critical fluid processing for phospholipid recovery is to develop purification techniques for these naturally-derived chemicals.

4. THE ROLE OF ANALYTICAL TECHNIQUES IN NUTRACEUTICALS

The analytical use of critical fluids spans over three decades of endeavour and applications

(Lee and Markides, 1990; Taylor, 1998) and there are many excellent tomes describing activity in this field (Luque de Castro et al., 1994; Dean, 1993). It is not the intention of this review to provide a detailed description of the use of analytical methodology for characterizing nutraceutical products, although such techniques as SFC can rapidly provide valuable information for the chemist and product formulator (King, 1990, 1998). There is no doubt that analytical SFC is a logical choice to characterize extracts or fractions obtained by critical fluid extraction and fractionation, but perhaps of more importance, is to describe options that exist for utilizing such analytical instrumentation to assist in optimizing and developing processing methods to produce nutraceuticals for the marketplace. The following is a summary of the information obtainable using analytical scale techniques:

- To indicate solubility or extractability of a compound
- For fractionating a natural product
- In support of process development
- For analysis of critical fluid-derived extract
- To deformulate a commercial product
- To determine required physicochemical data

With the advent of automated analytical SFE equipment, it has become possible to rapidly ascertain which extraction or fractionation conditions would be most relevant for scaling up the process. In the United States, analytical SFE instrumentation is produced by such firms as Isco, Applied Separations, Leco, and Jasco. Similarly, analytical scale SFC equipment is available from Berger Instruments, Gilson, Jasco, and Sensar. The equipment, which can be obtained from these vendors, can be if needed, slightly modified to study conditions that are amenable to processing nutraceuticals. King (1995) has provided a interesting review of how home-built equipment can be used for both analytical and process development purposes.

Two examples will be cited that show how analytical SFE instrumentation can be used to obtain information related to isolation of nutraceutical ingredients. Chandra and Nair (1996) have studied the extraction of isoflavone components from soya-based products, such as daidzein and genistein, using a manual SFE system. Neat SC-CO_2 proved relatively ineffective in removing the isoflavone components from the various soya-based matrices, however by simply varying the extraction conditions, it was found that 20 % (v/v) ethanol in SC-CO_2 at 50°C and 600 atm could remove over 90% of the isoflavones in <60 min. These survey extractions were performed on sample sizes ranging from 2-10 g, saving considerable time and labour in ascertaining what conditions were optimal for the extraction of these components. The need for relatively high pressures and cosolvent to remove the isoflavones from the matrix suggest that subcritical water extraction might be another alternative for isolating these nutraceutical components.

An automated SFE option has been utilized by Montanari et al. (1999) and Taylor and King (1998) to develop the conditions most amenable to isolating phospholipid concentrates from deoiled soy meal. In the former case, a set of conditions were tested by programming the automated SFE to run a large number of extractions on soy meal samples at various pressures, temperatures, and cosolvent (ethanol) levels. The results showed that at 70°C and 400 atm with 10 mole % ethanol phosphatidylcholine could be extracted preferentially relative to the other phospholipids in the soy meal. By utilizing higher pressures, it was found that the total amount of phospholipids extracted could be substantially increased at the expense of a slight reduction in of the phosphatidylcholine content of the final extract.

Similarly, an automated SFE unit was also used to ascertain what conditions were necessary to elute and separate phospholipid moieties from extracts obtained from SFE and other extraction methods (Taylor and King, 1998). In this case, the phospholipid-containing

extract was layered on top of a sorbent, such as alumina or silica gel, and elution and separation of the target compounds were assessed by changing the pressure, temperature, and quantity and composition of the cosolvent added to the fluid eluent. As summarized in Table 3, pure SC-CO_2 aided in removing the non-polar (triglyceride) components from the deposited sample, but the presence of a binary cosolvent (ethanol/water) with the SC-CO_2 proved necessary to affect elution of the phospholipids from the sorbent bed. By collecting various fractions during the stepwise elution from the column, while varying the conditions, various phospholipids could be enriched at the expense of others as indicated in Table 3.

Recently, using a similar approach, Taylor et al. (1999) have been able to produce concentrates enriched in phospholipids for the nutraceutical market. Table 4 summarizes the relative amounts of phospholipids in the initial SFE isolation and in the fractions obtained after SFC. Here the major components in the natural product (soybean oil triglycerides), were initially reduced by performing SFE with neat SC-CO_2, followed by sequential SFE/SFC utilizing SC-CO_2/cosolvent mixtures on the lecithin-containing residue remaining after SC-CO_2 extraction. By using SC-CO_2/ethanol/water fluid phases, one could not only perform preparative SFC for enriching and separating the phospholipids, but also the residual protein meal is devoid of any objectionable solvent residues. It should be noted that by selective density and composition programming of the fluid phase, coupled with time-based collection of eluent fractions, it is possible to isolate pure phospholipids for nutraceutical use.

A large number of experiments could be run in several days, and even over night on the automated SFE module, saving considerable cost and effort before scaling up the SFE/SFC process to a preparative level. Using such an approach, similar experiments have been run on other natural product matrices to assess whether comminution of the sample was necessary prior to conducting selective SFE for specific components, and what part of a natural plant,

containing the target components of interest, should be extracted (Taylor et al., 1994).

As was mentioned previously, analytical SFC could be of considerable aid in evaluating the content of nutraceutical-containing extracts. This is particularly true if one is looking for a rapid analysis method for quality control of selected ingredients, or to monitor differences in processing and raw materials. The elution order of lipophilic solutes is well known in capillary SFC (King and Snyder, 1997) and permits the separation of key lipophilic nutraceutical components. For example, in Figures 10a and b, the high resolution separation of fatty acids, squalene, tocopherols, and various phytosterols is illustrated using both flame ionization detection (FID) and a tandem SFC-mass spectrometer (SFC/MS) system (Snyder et al., 1993). There is considerable information here revealed to the analytical chemist on the chemical composition of the samples of deodorizer distillate and antioxidant sample, respectively considering the sample preparation is minimal and the analysis time is under 45 minutes.

On the subject of sample preparation, analytical SFC can save the analyst considerable time as illustrated in Figure 11. Here a nutraceutical capsule, a fish oil concentrate, has been characterized by capillary SFC (King, 1990) without resorting to any sample preparation. In this case, the oily nutraceutical formulation was exuded from the capsule and diluted with a minimal of hexane and directly injected into the chromatograph. By employing density programming, one can easily separate the tocopherol and sterol from the fish oil triglycerides containing ω-3 fatty acids. Recently, a similar approach has been used by us and others (DeSwaef et al., 1996) for characterizing saw palmetto extracts.

5. POTENTIAL FOR THE FUTURE

In summary, we have attempted in this review to provide some understanding of the basic concepts involved in the use of critical fluids, and to explain how these fluids are now

exploited for the production of nutraceutical and other naturally derived products. Several illustrative examples have been provided of processing concepts and equipment, from the laboratory scale through production plants. There now exists a thirty-year history of critical fluid processing technology upon which to draw, replete with many examples of components having nutraceutical value that have been extracted and fractionated in these dense fluids. The future of critical fluid technology is certainly promising especially in the rapidly growing field of nutraceuticals that require "natural processing" techniques.

As various by-product streams of conventional food processing operations are re-evaluated in light of the latest developments in nutraceuticals, it is becoming clearly apparent that many valuable components are lost in numerous waste streams as agricultural materials are highly refined to produce today's food products/ingredients. Thus, there is a concerted effort for the recovery of high-value components from such "waste" streams and critical fluid technology has a major role to play in this effort. For example, sources such as corn gluten meal, corn bran fibre and alfalfa leaf protein concentrate have been extracted with SC-CO_2 successfully for their pigment, sterol, and fatty acid content. The high tocopherol-sterol-squalene content of deodorizer distillate obtained during oil refining is well known and several schemes employing critical fluids have been reported for its fractionation to achieve higher purity materials (Ssuss and Brunner, 1999). Another processing agent that is a concentrator for nutraceutical components are the bleaching sorbents used by the vegetable oil processing industry. King and coworkers (King et al., 1992) have shown that very high oil yields can be obtained from clay bleaching earths, however the extract needs to be analyzed for enriched content of the nutraceutical components.

Another promising area for processing of nutraceuticals is conducting chemical or enzymatic reactions in supercritical media because enhanced reaction rates can be achieved

under supercritical conditions. Lipase catalyzed reactions of lipids such as esterification, interesterification or transesterification are especially attractive from nutraceuticals point of view since "designer" oils of desired fatty acid composition can be produced. Such processing can be coupled with supercritical extraction and fractionation steps where the starting material can be any oilseed but the product will be an oil of predesigned fatty acid composition.

Critical fluid technology offers versatility in process design and development where extraction using "green" solvents can be combined with fractionation and reaction steps in various ways to achieve the desired end product(s). In general, the overall flow diagram is quite simpler than conventional methods applied to reach the same goal. In addition, some of the new novel approaches for continuous handling of solid and liquid materials and equipment design are making critical processes much more efficient and competitive. Such systems can be designed with the flexibility to process different agricultural materials to give an advantage to the processor targeting the nutraceutical market.

For some product applications, critical fluid technology faces competition from conventional techniques such as molecular (vacuum) distillation, a time-honoured technique; although greater selectivity is potentially available utilizing the former methods. This coupled with the fact that CO_2-derived extracts exhibit in many cases extended shelf lives due to the prophylactic action of the residual, non-oxidative CO_2 atmosphere; as well as microbial destruction at higher pressures and temperatures, argues for a bright future for critical fluid technology in the nutraceutical marketplace.

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Table 1. Organizations processing or offering critical fluid-derived products

Flavex (Germany)	Fuji Flavor (Japan)
Hermsen (Germany)	Kobe (Japan)
HVG Barth (Germany)	Mori Oil Mills (Japan)
Kaffe HAG (Germany)	Ogawa (Japan)
SKW Trostberg (Germany)	Takasago (Japan)
KD - Pharma - IQA (Germany-Spain)	Takeda (Japan)
General Foods (United States)	Cultor (France)
John Haas (United States)	HITEX (France)
Praxair (United States)	Norac (Canada)
Yakima Chief (United States)	Aroma Tech OY (Finland)
Carlton United Breweries (United Kingdom)	Quest (Holland)
English Hops (United Kingdom)	Wells Investment Ltd. (New Zealand)
Steiner Hops (Germany-United Kingdom-United States)	

Table 2 - Enrichment factors of tocopherols from soybeans by SFE and SFE/SFC.

Tocopherol	SFE	SFE/SFC
alpha	4.33	12.1
beta	1.83	2.4
gamma	3.94	15.0
delta	3.75	30.8

Table 3 - SFC fractionation of lecithin on silica gel.

Fraction Collected	Eluent Parameters	Predominant Compounds
#1	350 bar, 50°C, CO_2	Triglycerides
#2	350 bar, 50°C, CO_2/M	"
#3	350 bar, 50°C, CO_2/M	
#4	500 bar, 50°C, CO_2/M	Phosphatidylethanolamine
#5	500 bar, 80°C, CO_2/M	Phosphatidylinositol + Phosphatidycholine
#6	500 bar, 80°C, CO_2/M	Phosphatidylcholine
#7	500 bar, 80°C, CO_2/M	Phosphatidylcholine

Fraction #2 modifier (M) is 10% ethanol:water (9:1)
Fractions #3-7 modifier (M) is 25% ethanol:water (9:1)

Table 4 - Relative amount of phospholipids from soybeans in SFE isolates and in SFC collected fractions.

Phospholipid	SFE [a]	SFC
Phosphatidylethanolamine	16.1	74.9
Phosphatidylinositol	9.2	20.8
Phosphatidic Acid	2.8	55.8
Phosphatidylcholine	15.6	76.8

All data in percent of that component
[a] relative to other eluting constitutents (oil and unidentified peaks)

Figure Captions:

Figure 1 - Phase diagram of water

Figure 2 - "Green" critical fluid processing options.

Figure 3 - Bench scale supercritical fluid extraction system.

Figure 4 - Semi-continous pilot plant extraction system.

Figure 5 - Thermal gradient supercritical fluid fractionation column.

Figure 6 - Porocritical continuous fluid extraction process.

Figure 7 - Separation scheme for potential nutraceutical components in soybeans.

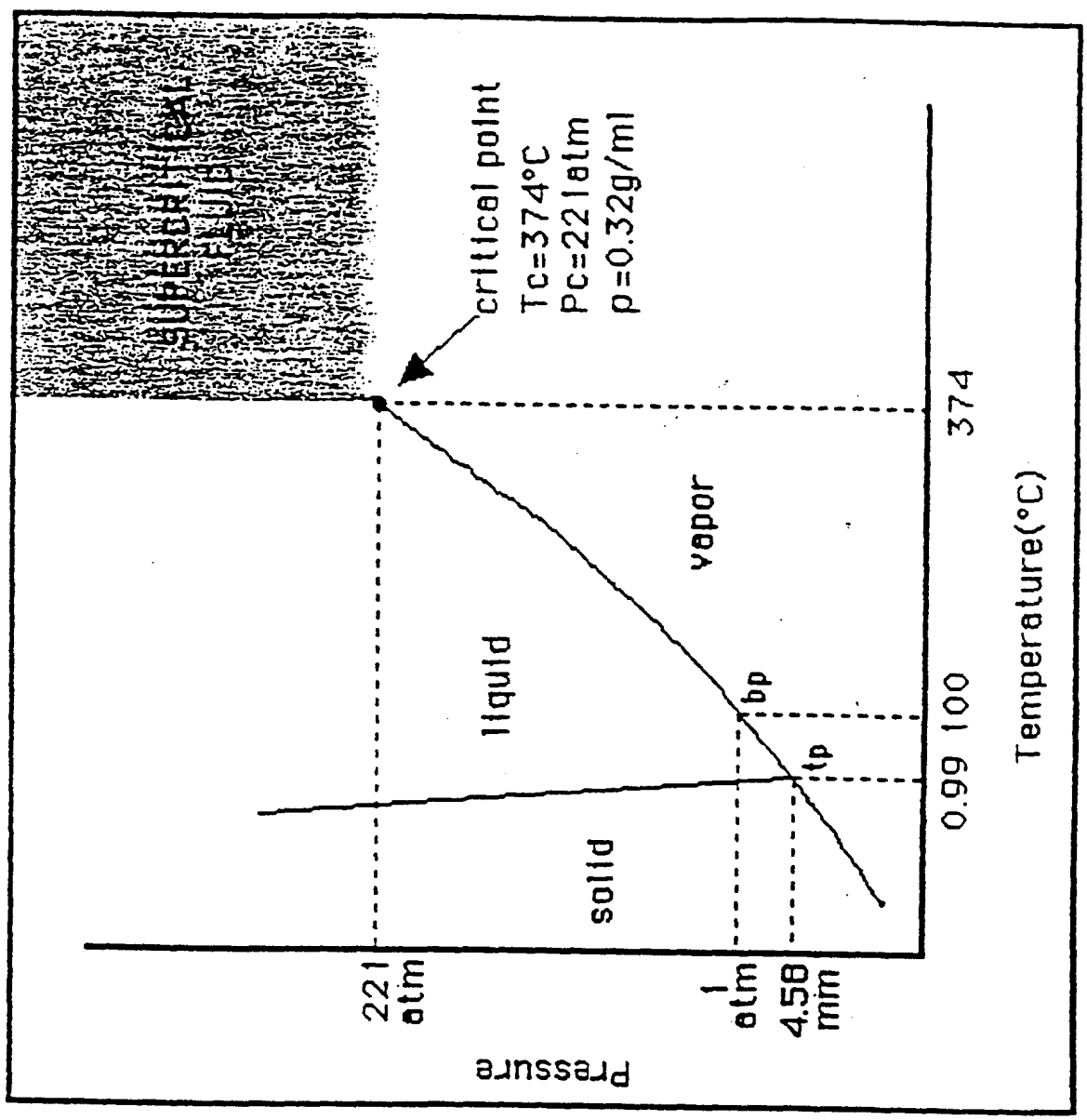
Figure 8 - Continuous countercurrent refining system.

Figure 9 - Tocopherol enrichment/fractionation by SFE/SFC technique.

Figure 10 - Capillary SFC characterization of potential nutraceutical component mixtures.

Figure 11 - Capillary SFC analysis of fish oil concentrate capsule.

Fig.1


SUPERCRITICAL
critical point
Tc=374°C
Pc=221atm
ρ=0.32g/ml
liquid
vapor
solid
bp
tp
221 atm
1 atm
4.58 mm
0.99 100
374
Pressure
Temperature(°C)

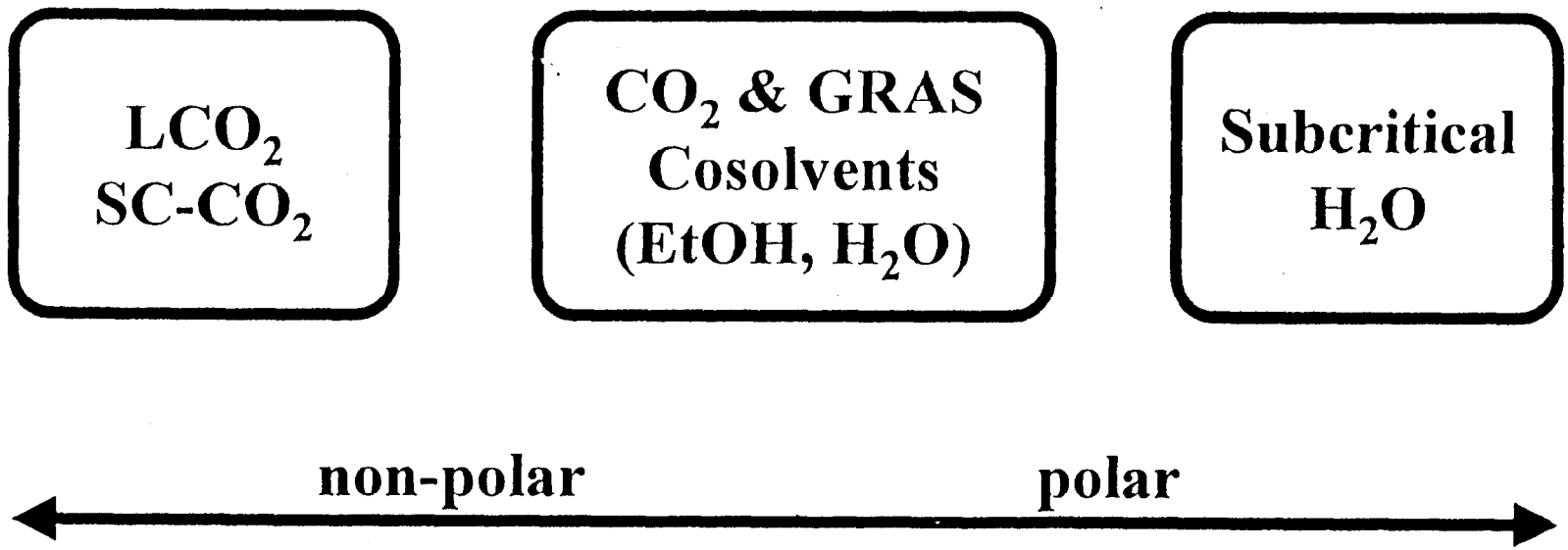

LCO_2
$SC\text{-}CO_2$
CO_2 & GRAS
Cosolvents
(EtOH, H_2O)
Subcritical
H_2O
non-polar
polar

Fig. 2

Fig. 3

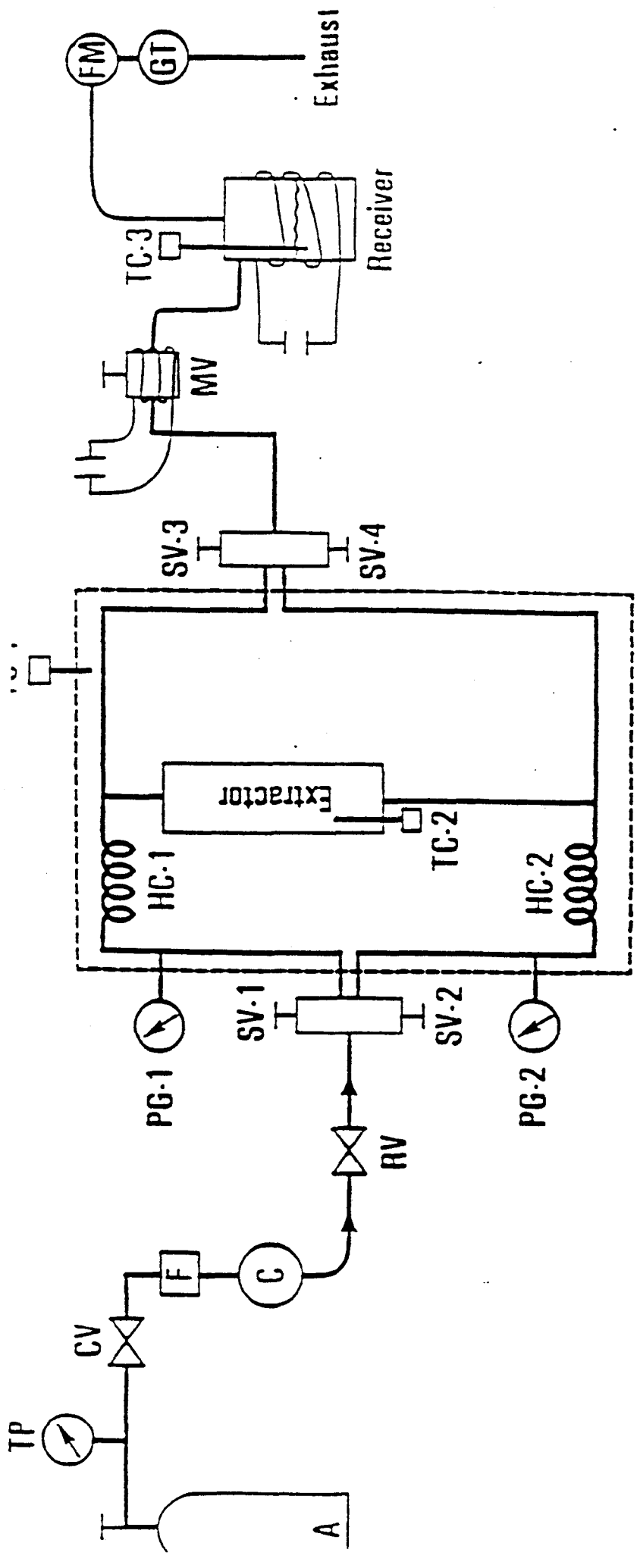

FM
GT
Exhaust
TC·3
Receiver
MV
SV·3
SV·4
Extractor
TC·2
HC·1
HC·2
SV·1
SV·2
PG·1
PG·2
RV
C
F
CV
TP
A

Fig 4

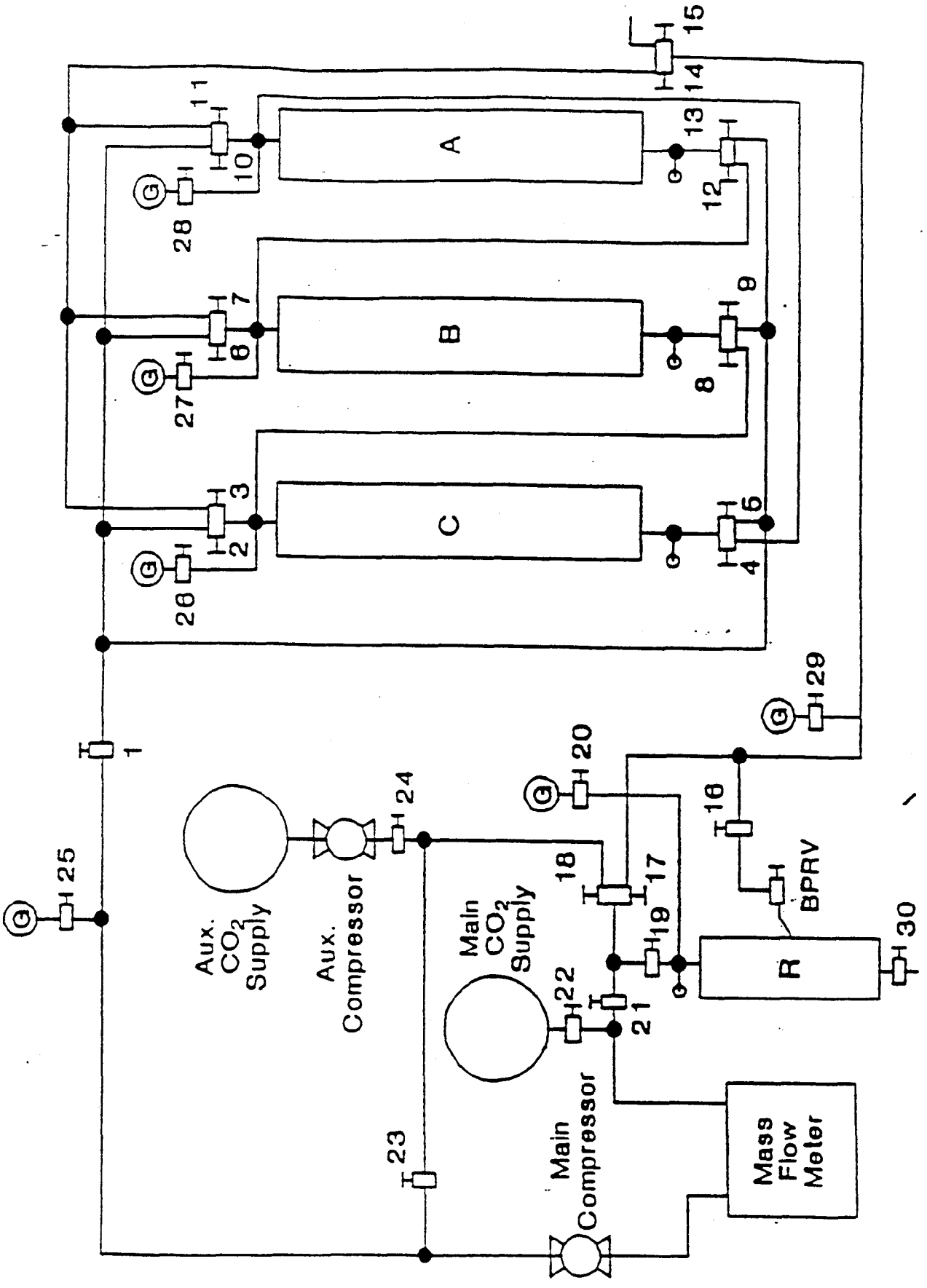

A
B
C
R
Aux. CO_2 Supply
Aux. Compressor
Main CO_2 Supply
Main Compressor
Mass Flow Meter
BPRV
1
2
3
4
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

Fig. 5

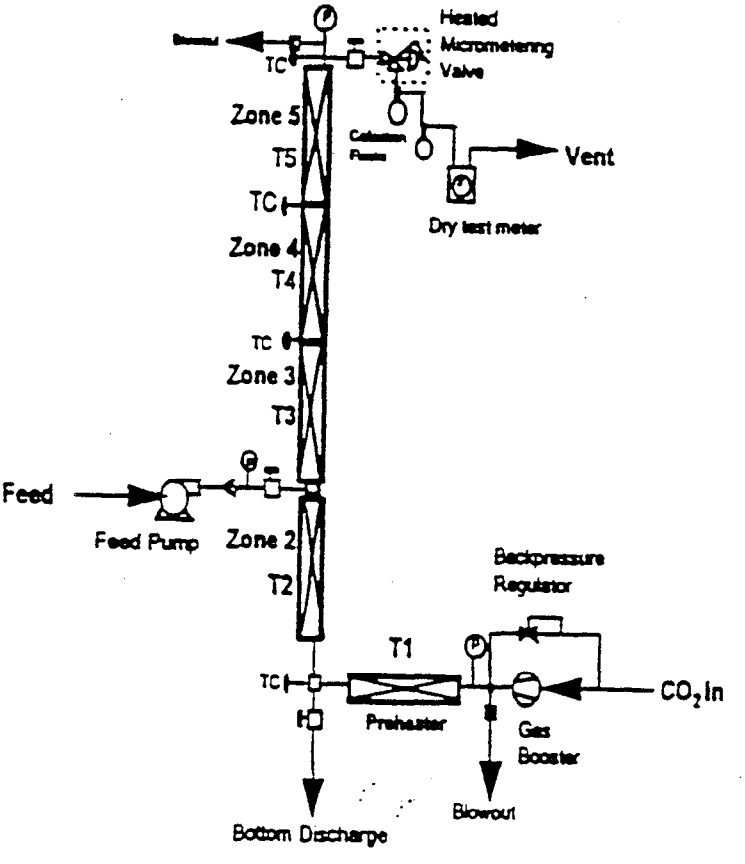
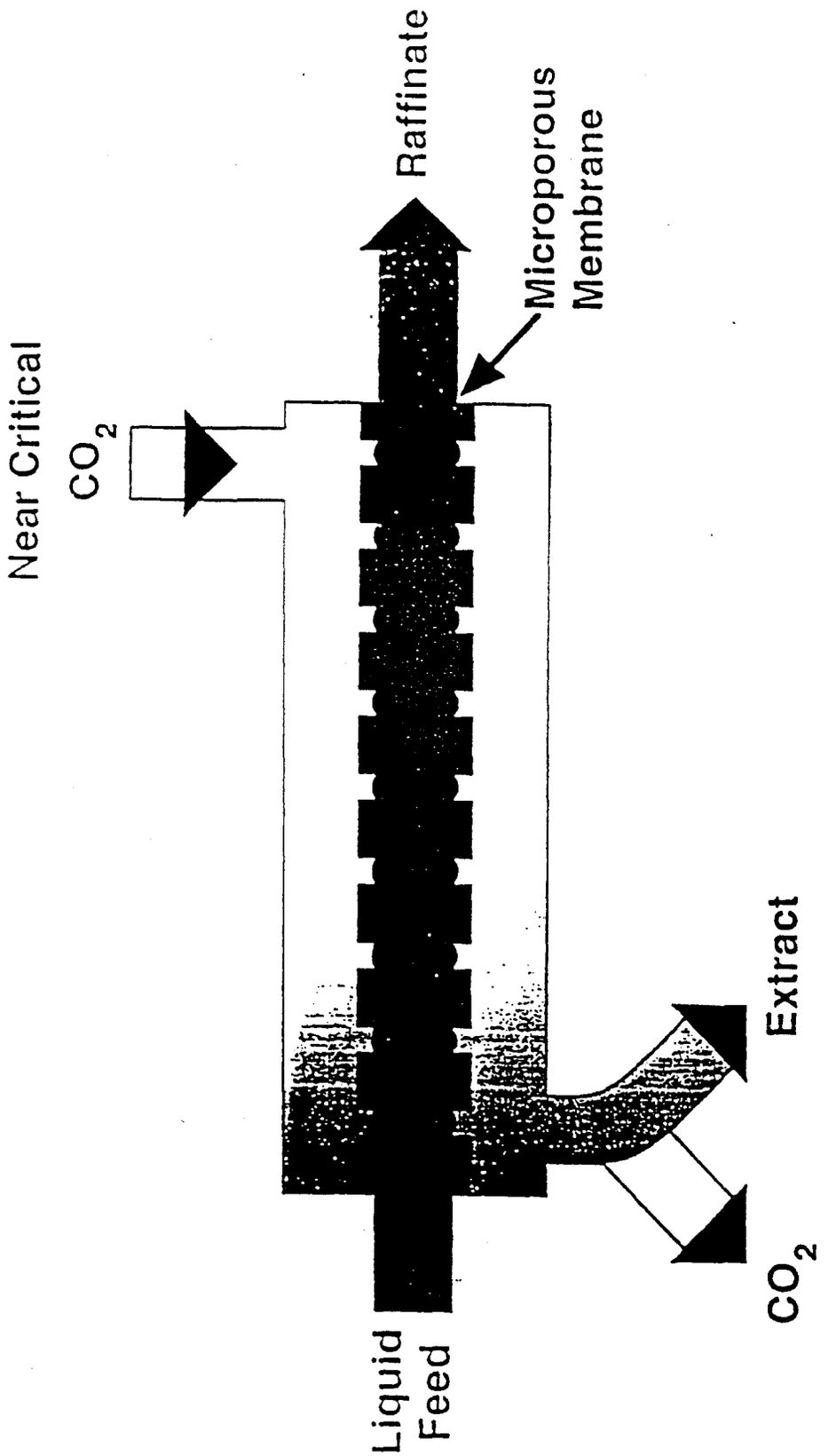

Heated
Micrometering
Valve
TC
Zone 5
T5
TC
Vent
Dry test meter
Zone 4
T4
TC
Zone 3
T3
Feed
Feed Pump
Zone 2
T2
Backpressure
Regulator
T1
TC
Preheater
CO_2 In
Gas
Booster
Blowout
Bottom Discharge

Fig. 6


Near Critical
CO_2
Raffinate
Microporous
Membrane
Extract
CO_2
Liquid
Feed

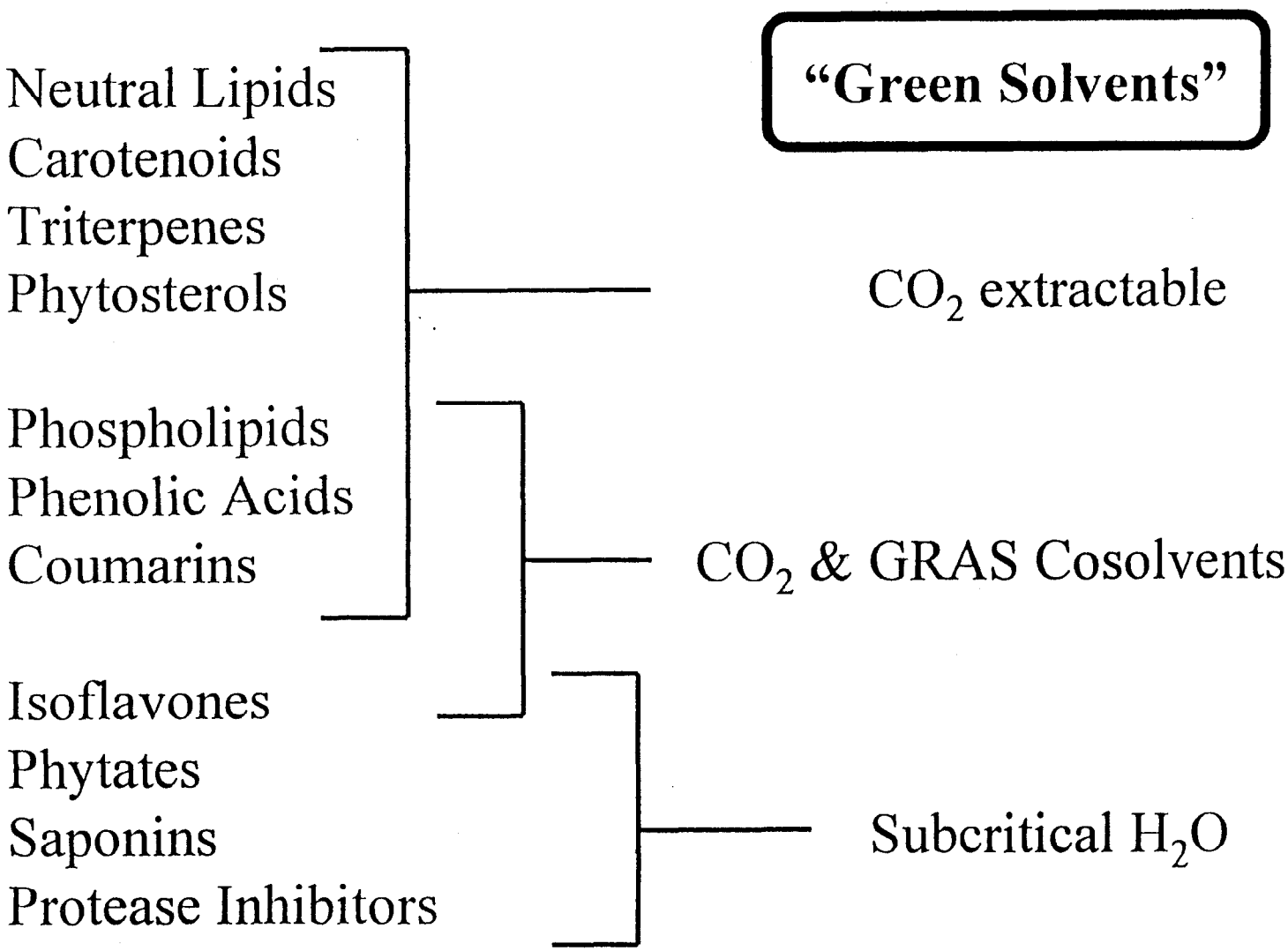
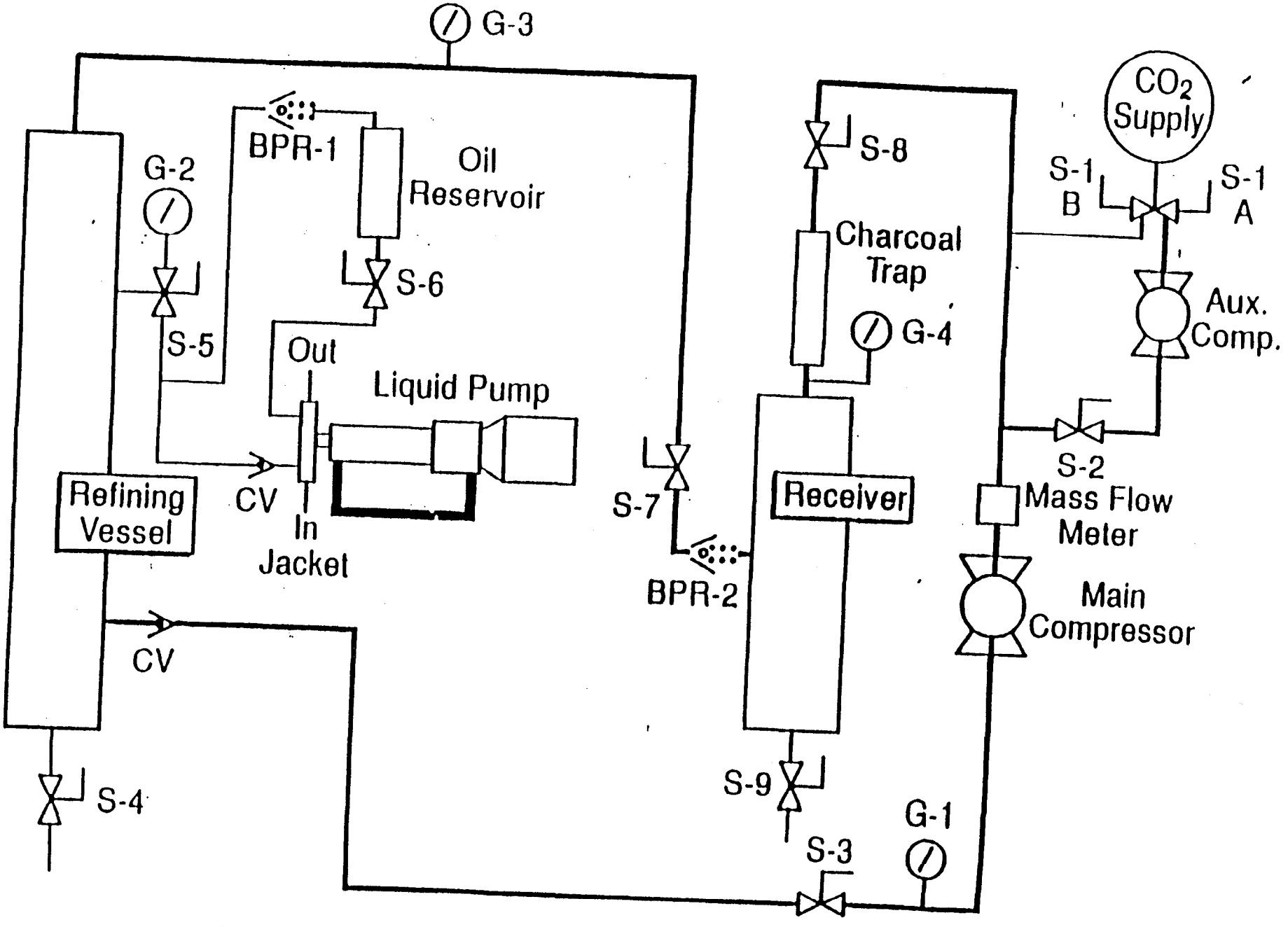

"Green Solvents"
Neutral Lipids
Carotenoids
Triterpenes
Phytosterols
CO_2 extractable
Phospholipids
Phenolic Acids
Coumarins
CO_2 & GRAS Cosolvents
Isoflavones
Phytates
Saponins
Protease Inhibitors
Subcritical H_2O

Fig. 7


G-3
BPR-1
G-2
Oil
Reservoir
S-6
S-5
Out
Liquid Pump
Refining
Vessel
CV
In
Jacket
CV
S-4
S-7
BPR-2
S-8
Charcoal
Trap
G-4
Receiver
S-9
S-3
G-1
CO_2
Supply
S-1
B
S-1
A
Aux.
Comp.
S-2
Mass Flow
Meter
Main
Compressor

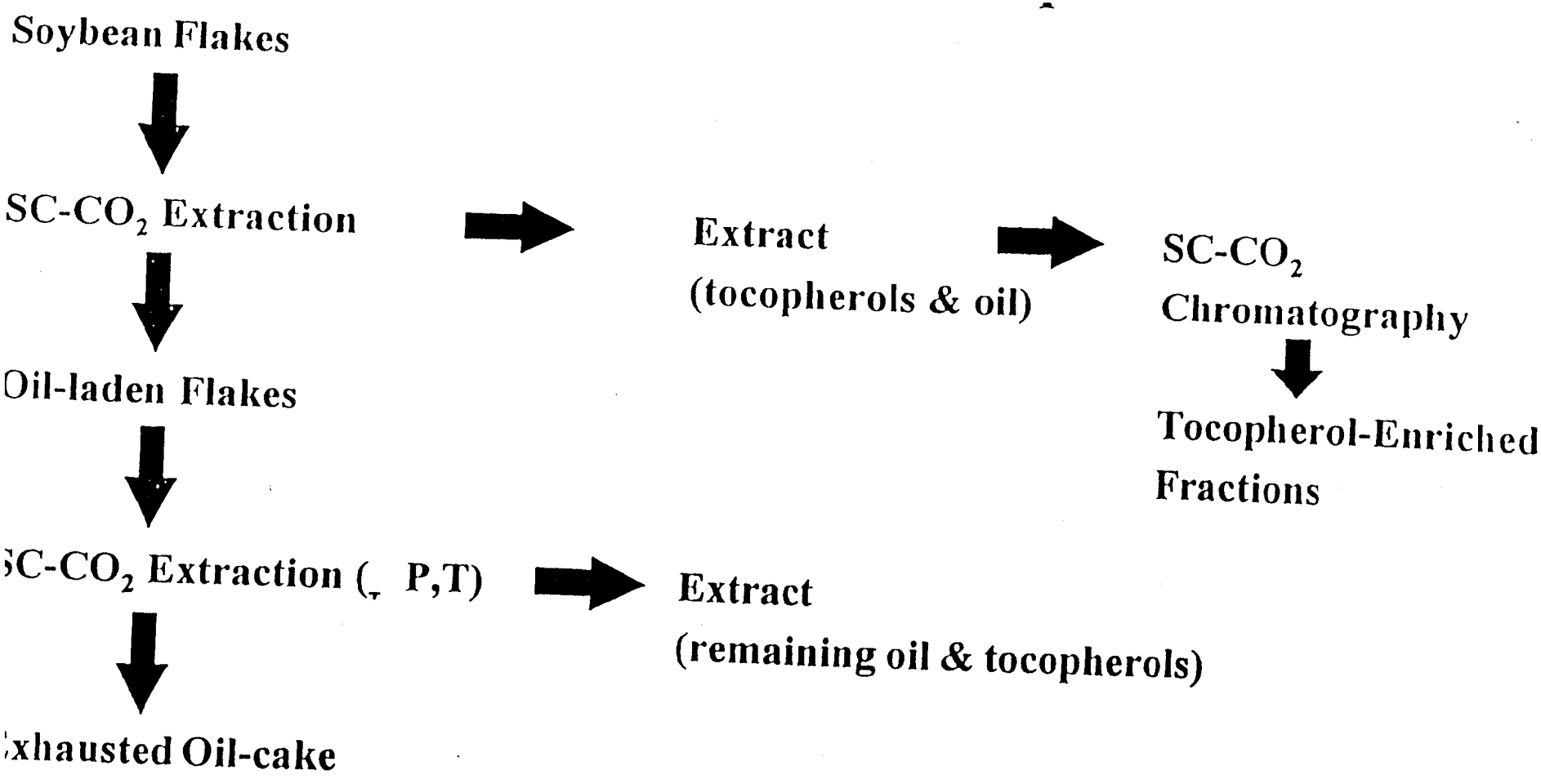
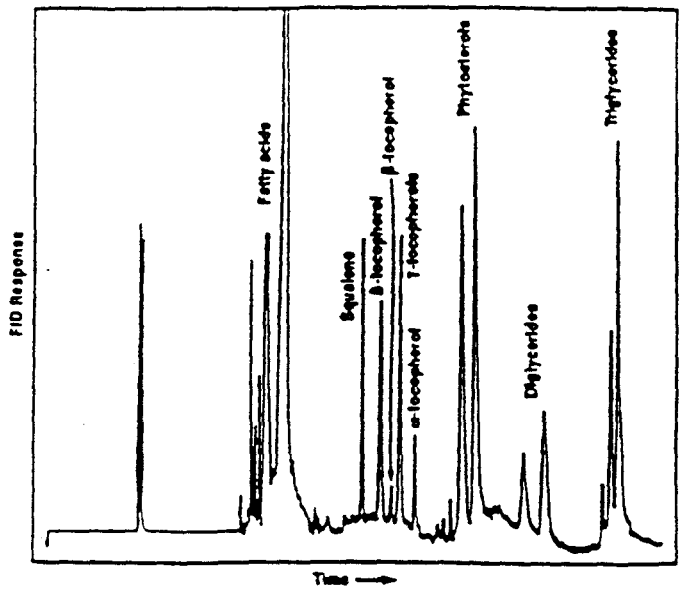

Soybean Flakes
SC-CO2 Extraction
Oil-laden Flakes
SC-CO2 Extraction (, P,T)
Exhausted Oil-cake
Extract
(tocopherols & oil)
SC-CO2
Chromatography
Tocopherol-Enriched
Fractions
Extract
(remaining oil & tocopherols)

Fig. 4

Fig. 1C


FID Response
Fatty acids
Squalene
δ-tocopherol
β-tocopherol
γ-tocopherols
α-tocopherol
Phytosterols
Diglycerides
Triglycerides
Time

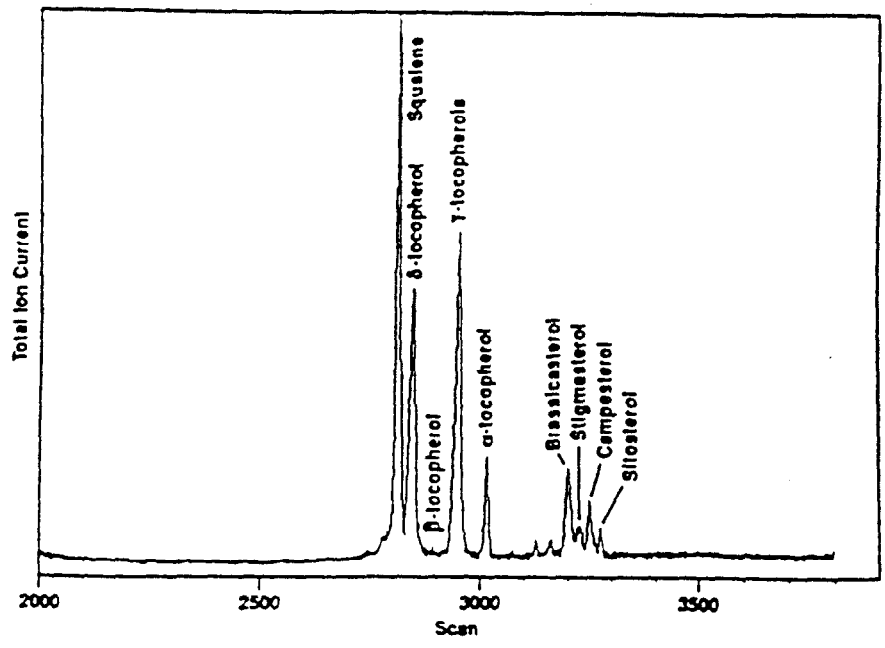
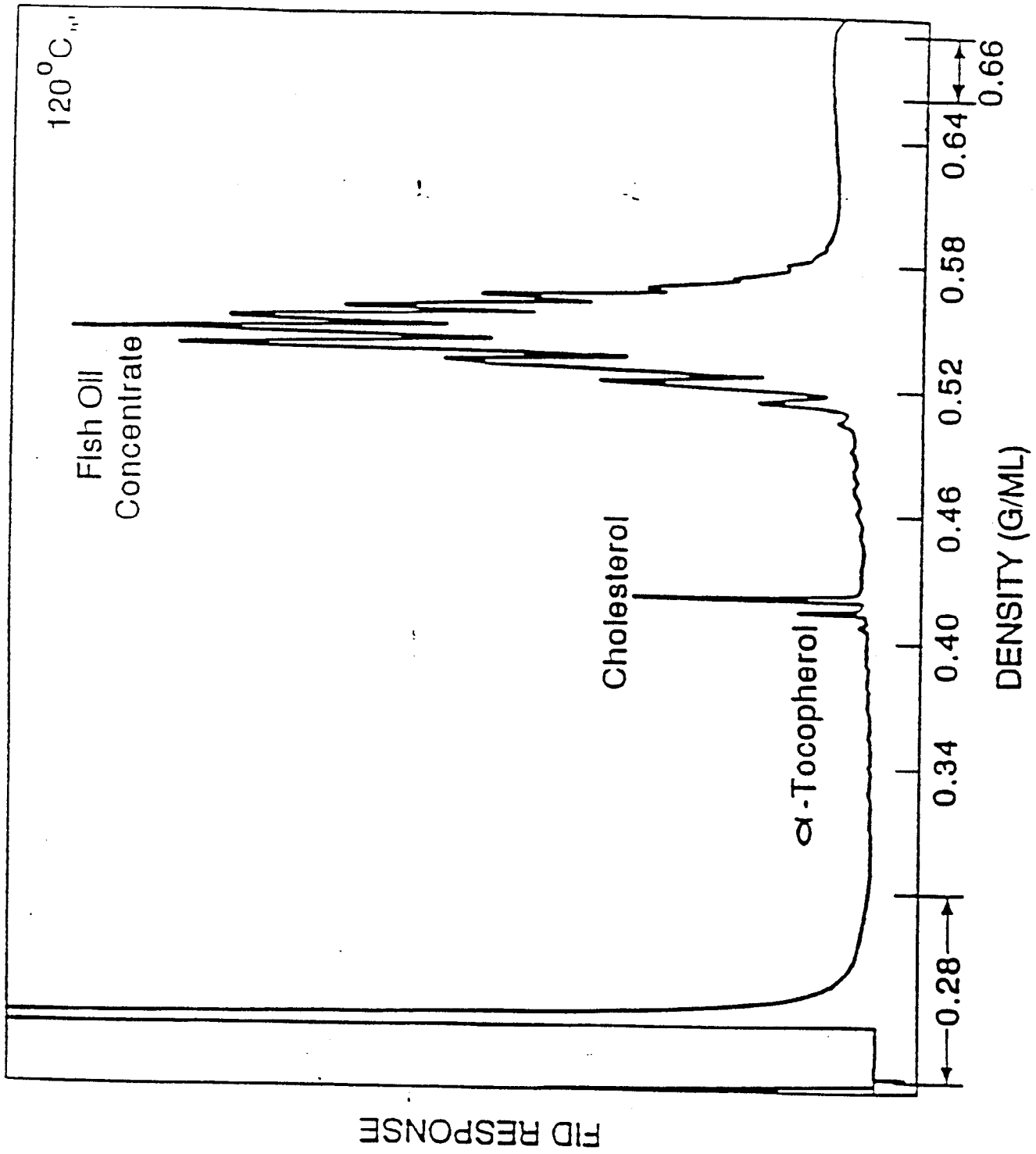

Total Ion Current
δ-tocopherol
Squalene
γ-tocopherols
β-tocopherol
α-tocopherol
Brassicasterol
Stigmasterol
Campesterol
Sitosterol
2000
2500
3000
3500
Scan

Fig.


120°C
Fish Oil Concentrate
Cholesterol
α-Tocopherol
0.28
0.34
0.40
0.46
0.52
0.58
0.64
0.66
DENSITY (G/ML)
FID RESPONSE